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# Implications of the Progressive Self-association of Wild-type Human Factor H for Complement Regulation and Disease

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Factor H (FH) is a major regulator of complement alternative pathway activation. It is composed of 20 short complement regulator (SCR) domains and is genetically associated as a risk factor for age-related macular degeneration. Previous studies on FH suggested that it existed in monomeric and dimeric forms. Improved X-ray scattering and analytical ultracentrifugation methodology for wild-type FH permitted a clarification of these oligomeric properties. Data at lower concentrations revealed a dependence of the X-ray radius of gyration values on concentration that corresponded to the weak self-association of FH. Global sedimentation equilibrium fits indicated that a monomer–dimer equilibrium best described the data up to 1.3 mg/ml with a fitted dissociation constant $K_D$ of 28 μM and that higher oligomers formed at increased concentrations. The $K_D$ showed that about 85–95% of serum FH will be monomeric in the absence of other factors. Size-distribution analyses in sedimentation velocity experiments showed that monomeric FH was the major species but that as many as six oligomeric forms co-existed with it. The data were explained in terms of two weak dimerisation sites recently identified in the SCR-6/8 and SCR-16/20 fragments of FH with similar $K_D$ values. These observations indicate a mechanism for the progressive self-association of FH and may be relevant for complement regulation and the formation of drusen deposits that are associated with age-related macular degeneration.



*Keywords:* factor H; oligomerisation; X-ray scattering; analytical ultracentrifugation; age-related macular degeneration

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In the human innate immune defence system, the central complement component C3 is activated to C3b by the cleavage and removal of the small anaphylatoxin C3a to initiate the alternative pathway in serum. C3b is regulated by factor H (FH) in order to prevent complement-mediated host cell damage, in which FH acts as a co-factor for factor I cleavage of C3b to form iC3b,[1–3] accelerating the decay of the C3 convertase C3bBb[2,4] and competing with factor B for binding to C3b.[5] FH is composed of 20 short complement regulator (SCR) domains, each of about 61 residues in length. SCR domains, also known as short consensus repeats, Sushi or complement control protein domains,[6] constitute the most abundant domain type in complement proteins. There are multiple binding sites for C3b within the 20 SCR domains,[7,8] and likewise there are multiple binding sites for heparin.[9–12] FH regulates surface-bound C3b activity by recognising charge (anionic) clusters on the surfaces of host cells that are mimicked by heparin. The initial contact with host cells is made through its C-terminal end, which is followed by N-terminal regulatory activity.[13,14] Polymorphisms and mutations in FH have been associated with age-related macular degeneration (AMD),[15–19] the most common cause of blindness in the elderly in the Western world, and with atypical haemolytic uraemic syndrome (aHUS),[19,20] a rare

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Abbreviations used: FH, factor H; SCR, short complement regulator; $R_G$, radius of gyration; AMD, age-related macular degeneration; aHUS, atypical haemolytic uraemic syndrome.

disease leading to renal failure that affects individuals of all ages but primarily children and young adults†. The involvement of FH indicates that complement activation and regulation in the retina and the kidney endothelium have been impaired.

Intact FH has not been crystallised to date for reasons of its size, glycosylation and inter-SCR flexibility. Thus, solution scattering and electron microscopy methods have been applied to show that the FH SCR domain structure is not fully extended in solution.[21–23] X-ray scattering and ultracentrifugation, in combination with constrained scattering modelling, are powerful approaches[24,25] that resulted in the first molecular structures for FH and its SCR-6/8, SCR-1/5 and SCR-16/20 fragments.[23,26,27] Homology models for the SCR domains provided many of the first structural explanations for AMD and aHUS.[23,28,29] Thus, AMD was associated with a common Tyr402-His polymorphism, which is located at the surface of SCR-7 and close to a heparin binding site. The aHUS-related mutations cluster at the surface of SCR-20 and to a lesser degree in SCR-16 to SCR-19, and they are associated with another heparin binding site. The distribution of more than 100 genetic alterations† showed that the observed clinical phenotype is correlated with their structural location.[19] NMR and crystal structures, starting with those for SCR-5 and SCR-15/16 and more recently extended to those for SCR-6/8 and SCR-19/20, have confirmed and extended these predictions.[6,12,30–33] Even with these structures, there is no clear indication of a molecular mechanism involving FH that leads to AMD.

One of the hallmarks of AMD is the appearance of drusen, an amyloid plaque-like deposition in Bruch's membrane, a layer interposed between the retinal pigment epithelium and the choroidal vasculature.[34,35] The deposits contain oxidized lipids and many aggregated proteins, including FH.[36] The self-association properties of FH are thus relevant to drusen formation. FH was originally shown to be monomeric by ultracentrifugation.[37] Dimeric FH was demonstrated by scattering, but this observation could not be subsequently replicated.[21,23] Partial FH SCR-15/18 and SCR-15/20 dimers were however observed by non-reducing SDS-PAGE, and SCR-1/7 was observed to interact with SCR-1/20 by surface plasmon resonance.[8,13] Our recent ultracentrifugation and scattering studies showed that SCR-6/8 and SCR-16/20 (but not SCR-1/5) exhibit weak monomer–dimer associations.[26,27] These studies indicate at least two potential dimerisation sites in FH. In combination, the two sites would constitute a mechanism for the continual self-association of FH that would lead ultimately to aggregate formation. In this study, we re-investigated the oligomerisation properties of native FH. Through the use of improved scattering and ultracentrifugation instrumentation and analyses, we found and here show that FH exhibits a monomer–dimer equilibrium at physiological concentrations and multiple oligomers at higher concentrations. We discuss the implications of this result for complement regulation and AMD.

† http://www.fh-hus.org

## X-ray scattering of FH oligomers

The purification of wild-type FH for X-ray scattering and analytical ultracentrifugation experiments utilised a 3-l pool of just-outdated anonymised human plasma from the Royal Free Hospital Blood Bank with an anti-FH monoclonal antibody Sepharose MRC-OX23 column as previously described.[23,38] The final column eluate in 3 M $MgCl_2$ was dialysed into Hepes buffer [10 mM Hepes, 137 mM NaCl and 0.5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4]. The FH concentration step at 4 °C employed a gentle centrifugation approach without stirring (Amicon® Ultra-15 centrifugal filter devices with a molecular-mass cutoff of 50 kDa at 2500*g*). Non-specific aggregates of FH and human serum albumin were removed by gel filtration on a Superose™ 6 prep grade XK 16/60 column, and the sample was re-concentrated by centrifugation. All FH samples were checked using SDS-PAGE before and after scattering and ultracentrifugation experiments. An absorption coefficient of 16.7 (1%, 280 nm, 1-cm path length) was used to determine concentrations.[23]

The X-ray scattering radius of gyration ($R_G$) of FH monitors its degree of elongation. FH prepared 2 days beforehand was studied on Instrument ID02 at the European Synchrotron Radiation Facility[39] in eight concentrations between 8.7 and 0.43 mg/ml in Hepes buffer (Fig. 1). Improved Guinier $R_G$ fits were obtained at lower $Q$ values compared with the previous X-ray measurements at the Synchrotron Radiation Source at Daresbury ($Q = 4\pi \sin\theta/\lambda$, $2\theta$ = scattering angle, $\lambda$ = wavelength).[21,23] For reason of the higher beam intensities at the European Synchrotron Radiation Facility, improved signal–noise ratios were obtained (Fig. 1a and b). There is better control of radiation damage on Instrument ID02 as this is monitored online during data acquisition. The Guinier $R_G$ and its cross-sectional radius of gyration ($R_{XS\text{-}1}$) parameters (but not its $R_{XS\text{-}2}$ parameter: not shown) and the associated intensity $I(0)/c$ parameters showed small but visible concentration dependences (Fig. 1c–e). The $R_{XS\text{-}2}$ parameter differs from SCR protein to protein and generally monitors the averaged short-range degree of bend between two or among three adjacent SCR domains along the length of the protein.[23] An additional medium-range proximity relationship between non-neighbouring SCR domains that are further apart in the sequence leads to the observation of the $R_{XS\text{-}1}$ region.[23] This weak concentration dependence showed that FH oligomerisation had occurred. Data obtained using phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$ and 1.15 mM $KH_2PO_4$, pH 7.4) were consistent with the Hepes data (Fig. 1c and e). The $R_G$ value extrapolated to zero concentration is 8.90 ± 0.19 nm, while $R_{XS\text{-}1}$ is 2.51 ± 0.06 nm and $R_{XS\text{-}2}$ is 1.79 ± 0.01 nm. If the slope in Fig. 1d corresponds to a